High Purity RNA from Plants

Isolating high quality RNA from plant tissue can be problematic due to the presence of polysaccharides, humic acid, polyphenols and tannins. A well-tested solution to these difficulties is accomplished with a simple modification of the MasterPure™ Complete DNA & RNA Purification Kit. The procedure employs a straightforward cell lysis while inactivating endogenous ribonucleases, followed by a rapid desalting procedure to remove contaminating molecules without the use of toxic organic solvents. The recovered RNA can be used for many applications including amplification, hybridization, RNase protection, and RT-PCR. For a detailed, modified protocol, please see the attached document or contact Lucigen Technical Support.

RNA from Plants Protocol

Use reagents from the MasterPure Complete DNA & RNA Purification Kit. Users will need to supply the following:

RNase-Free water

100 mM DTT

Lysis of Plant Tissue Samples

Thoroughly mix all solutions to ensure uniform composition before use.

1. Collect 25-100 mg of fresh or frozen plant tissue and homogenize by quick-freezing in liquid nitrogen and grinding to a fine powder with a prechilled mortar and pestle. Other tissue homogenization methods can also be used.
2. Allow the liquid nitrogen to dissipate, but do not allow the sample to thaw.
3. To each sample, quickly add: 600 µL Tissue and Cell Lysis Solution, 6 µL 100 mM DTT, 1 µL Proteinase K
4. Mix by vortexing vigorously for 1 minute.
5. Incubate at 56°C for 15 minutes. Mix by vortexing every 5 minutes for 15-30 seconds to improve the yield of nucleic acids. ***Note:*** *For some tissues, shortening this step may be acceptable.*
6. Pellet the debris by centrifugation for 5 minutes at ≥10,000 x g at room temperature and transfer the clarified supernatant to a new tube. Minimize the carryover of particulates.
7. Place the samples on ice for 3-5 minutes.

Precipitation of Nucleic Acids

1. Add 250 µL of MPC Protein Precipitation Reagent to the sample and mix by vortexing vigorously for 10 seconds.
2. Pellet the debris by centrifugation at 4°C for 10 minutes at ≥10,000 x g in a microcentrifuge.
3. Transfer the supernatant to a clean microcentrifuge tube and discard the pellet.
4. Add 500 µL of isopropanol to the recovered supernatant. Invert the tube 30-40 times.
5. Pellet the nucleic acids by centrifugation at 4°C for 10 min. at ≥10,000 x g in a microcentrifuge.
6. Carefully pour off the isopropanol without dislodging the total nucleic acid pellet.
7. Remove all of the residual isopropanol. ***Note:*** *The pellet may not be white at this step.*

Removal of Contaminating DNA from RNA Preparations

1.Prepare 200 µL of DNase I solution for each sample as follows:

173 µL RNase-Free Water
20 µL 10X DNase Buffer
5 µL RNase-Free DNase I
2 µL RiboGuard RNase Inhibitor

2. Completely resuspend the nucleic acid pellet in 200 µL of DNase I solution.
3. Incubate at 37°C for 10 minutes. Note: Additional incubation (up to 30 minutes) may be necessary to remove all contaminating DNA
4. Add 200 µL of 2X T and C Lysis Solution; mix by vortexing for 5 seconds.
5. Add 200 µL of MPC Protein Precipitation Reagent; mix by vortexing 10 seconds; place on ice for 3-5 minutes.
6. Pellet the debris by centrifugation at 4°C for 10 minutes at ≥10,000 x g in a microcentrifuge.
7. Transfer the supernatant containing the RNA into a clean microcentrifuge tube and discard the pellet.
8. Add 500 µL of isopropanol to the supernatant. Invert the tube 30-40 times.
9. Pellet the purified RNA by centrifugation at 4°C for 10 minutes in a microcentrifuge.
10. Carefully pour off the isopropanol without dislodging the RNA pellet.
11. Wash twice with 70% ethanol. Centrifuge briefly. Remove all of the residual ethanol with a pipet.
12. Resuspend the RNA in 10-40 µL of RNase-Free Water.
13. Add 1 µL of RiboGuard RNase Inhibitor (optional). Store at –70°C.